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Human Salivary Sugar Clearance after Sugar Rinses and Intake of Foodstuffs

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Key Words
Carbohydrate · High-performance ion-exchange chromatography · Pulsed amperometric detection · Salivary clearance · Sugars

Abstract
The aetiology of dental caries is in part related to the retention time of dietary carbohydrates in the oral cavity and their subsequent metabolism by the oral bacteria. Salivary clearance of fermentable carbohydrates from three different foodstuffs was examined in 5 subjects and analyses performed by high-performance anion-exchange chromatography with pulsed amperometric detection. The clearance of glucose, fructose, sucrose, maltose and sorbitol rinses was studied as well as that of chocolate bars, white bread and bananas. Of the sugar rinses studied, sucrose was removed from saliva most rapidly whilst appreciable levels of sorbitol remained even after 1 h. Clearance of residual carbohydrates from bananas and chocolate bars seemed marginally faster than in the case of bread, but sucrose levels still tended to fall more quickly than other carbohydrates studied. Surprisingly, carbohydrate residues from the three foods studied were still present in the mouth even 1 h after ingestion, which is longer than has hitherto been reported.

Dental caries is initiated by the consumption of fermentable carbohydrates which are converted into organic acids by bacteria in dental plaque. The rise in acidity can cause demineralisation. Since cariogenic effects are, in part, related to the retention time of carbohydrates in the mouth as a result of their acidogenicity, the role of saliva in oral carbohydrate clearance is of primary interest. The salivary clearance of sugars is influenced by the properties of the foodstuff [Lundqvist, 1952], the amount of ingested carbohydrate [Goulet and Brudevold, 1984], the sampling site in the mouth [Britse and Lagerlöf, 1987; Strong et al., 1987] and physiological factors such as salivary flow rate and the volume of saliva in the mouth before and after swallowing [Dawes, 1983; Lagerlöf et al., 1987].

Analytical methods for the determination of carbohydrates in foods and biological fluids include enzymatic methods [Hase et al., 1987; Lindfors and Lagerlöf, 1988; Weatherell et al., 1989] and chromatographic techniques including paper chromatography and thin layer chromatography [Carlson, 1968; Anumula and Spiro, 1983]. High performance ion-exchange chromatography combined with pulsed amperometric detection (HPIEC-PAD) provides the most selective and sensitive method currently available for analysing complex samples containing carbohydrate-containing samples such as saliva [Gough et al., 1996]. The procedure can be used on small sample volumes (50 μl) and can simultaneously determine the level of more than one carbohydrate in any given sample. Using this technique we have examined the clearance from saliva of the carbohydrates glucose, fructose, sucrose, maltose and sorbitol after a mouth rinse and the clearance of relevant sugars after eating carbohydrate containing-foods.

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Materials and Methods

Materials and Experimental Subjects

All chemicals were either AnalaR grade from BDH-Merck, Poole, Dorset or of the highest grade available from the Sigma-Aldrich Company Ltd., Poole, Dorset, UK. Water was of the AnalaR grade with a maximum specific conductance at 20 °C of 0.05 μScm^{-1}. Five healthy dentate volunteers, 3 males and 2 females, participated in this study.

Foodstuffs Studied

Mouth Rinses. Sucrose, glucose, fructose, maltose and sorbitol solutions were administered as 10-ml mouth rinses at concentrations of 10% (w/v). These solutions were rinsed round in the mouth for 1 min and then expectorated.

Bread. Plain white sliced bread was bought fresh and frozen at –20 °C. A normal bite size of 10 g was used in all experiments.

Chocolate Bar. Chocolate-nougat bars supplied by Mars Confectionery Ltd., Slough, Bucks, UK were stored frozen at –20 °C. A bite size piece (10 g) from the centre of the bar was used in all experiments.

Banana. Bananas were purchased at stage 4 of ripening and supplied at stage 5 (see Fyffes colour index chart, Fyffes Group Ltd., Basingstoke, Hants, UK). A bite size of 10 g was used in all experiments.

Sampling of Saliva

Sampling of saliva was carried out at the same time of day, approximately 11 a.m., and after 2.5 h of fasting. Before the intake of each foodstuff to be studied, a fasting saliva sample was collected from each individual. At the start of the test session, subjects were asked to rinse or chew the test food in a manner described under the food testing protocol outlined in the Scientific Consensus Conference [1986]. After 1, 3, 5, 8, 15, 30 and 60 min, whole saliva was dribbled for 1 min into a vial on ice containing NaF adjusted to give a final concentration of 0.1% (w/v) [Gough et al., 1996]. Samples were frozen at –20 °C immediately after collection and analysed within 10 days.

Sample Preparation

Prior to analysis, saliva samples were thawed and clarified by centrifugation at 10,000 *g* for 10 min at 4 °C (Microspin 12S Sorvall Instruments, Dupont). Samples (0.25 ml) were diluted to 5 ml with water and passed through a hydrophobic filter cartridge (Dionex OnGuard-RP cartridge) to remove particulate matter, proteins and inorganic substances.

Analysis

The system used for HPIEC-PAD consisted of a Dionex (Camberley, Surrey, UK) BioLC model 4000i gradient pump and model PAD 2 detector. Sample injection was via a micro-injection valve equipped with a 50-µl sample loop. A column (3×250 mm) of Dionex HPIC-AGG pellicular anion exchange resin and a Carbo Pac PA guard column (3×25 mm) were used in all experiments. The eluent was 150 m*M* NaOH prepared by dilution of carbonate-free 50% (w/v) NaOH solution with water. Detection was by pulsed amperometric detector [Johnson and La Course, 1990] using a gold working electrode and triple pulse amperometry. The following pulse potentials and durations were used for analysis: $E_1 = 0.05$ V ($t_1 = 120$ ms), $E_2 = 0.65$ V ($t_2 = 60$ ms), $E_3 = -0.95$ V ($t_3 = 240$ ms). Data were collected and peak areas integrated on a Trio chromatography computing integrator (Trivector, Bedfordshire, UK). The methodology has been presented in detail by Gough et al. [1996].

Table 1. Performance characteristics of the HPIEC procedure

Sugar	Retention time min		% RSD		Sensitivity (µg/ml)	Correlation coefficient	
	A	B	C	D		A	B
Sorbitol	5.12	4.65	3.0	5.0	3.0	0.99	0.97
Glucose	8.57	6.22	3.5	5.7	0.3	0.99	0.99
Fructose	9.78	6.92	5.8	2.5	0.3	0.99	0.99
Sucrose	11.75	8.48	2.6	4.8	0.3	0.80	0.99
Maltose	21.47	15.25	2.2	2.6	3.0	0.99	0.99

A = Standard solutions 10–250 µg/ml, detector PAD at lowest sensitivity range 30,000 nA;
B = standard solutions 0.03–20 µg/ml, detector PAD at highest sensitivity range 1,000 nA;
C = percentage relative standard deviation (% RSD) for a high sugar concentration (250 µg/ml) detected at 30,000 nA (n = 5);
D = % RSD for a low sugar concentration (5 µg/ml) detected at 1,000 nA (n = 5).

Results

Salivary Sugar Analysis

Standard solutions containing a mixture of glucose, sucrose, fructose, maltose and sorbitol, 0.03–250 µg/ml of each sugar, were prepared. Aliquots (50 µl) of each standard solution were analysed and a standard curve for each component was produced utilising the appropriate detector output range (table 1). All sugars were clearly separated and the correlation between peak area and sugar concentration was linear.

Comparison of Rinses

Figure 1 shows the salivary clearance curves from each of the 5 subjects for the five carbohydrate rinses studied. The fasting concentrations of the five sugars together with the levels after 60 min are shown in table 2. Individual subjects showed a predisposition to either rapid or slow clearance irrespective of the carbohydrate. Subjects 1 and 2 had the fastest clearance rate, whilst 3 and 5 were intermediate and 4 was generally the slowest. Following a glucose rinse the salivary glucose concentrations had reached fasting levels in only 2 of the 5 subjects after 1 h and in 1 of these 2 cases, the level was below the fasting level. The loss of fructose from saliva is similar to glucose, but all levels remained well above fasting levels (which were below the detection limit) even 1 h after the rinse. Sucrose was cleared from the saliva the most rapidly and concentrations had fallen to less than 0.3 µg/ml (the lowest level detectable) in 3

Table 2. Sugar concentrations in saliva (µg/ml) from 5 individuals 60 min after a sugar rinse with a 10% (w/v) solution

Subject	Glucose	Sucrose	Fructose	Maltose	Sorbitol
1	32±0.5 (27.5±0.15)	<0.3 (<0.3)	1.23±0.04 (<0.3)	<3.0 (<3.0) 61±2.7[a]	6.0±0.07 (<3.0)
2	1.1±0.1 (1.3±0.03)	<0.3 (<0.3)	1.0±0.04 (<0.3)	<3.0 (<3.0) 11±0.7[a]	12.0±0.1 (<3.0)
3	14.9±0.02 (25.3±0.5)	<0.3 (<0.3)	7.0±0.2 (<0.3)	<3.0 (<3.0) 6.0±0.14[a]	30.0±0.1 (<3.0)
4	50.0±1.4 (32±0.3)	<0.3 (<0.3)	14.0±1.3 (<0.3)	<3.0 (<3.0) 50±0.25[a]	933±32.5 (<3.0)
5	9.2±0.4 (3.4±0.1)	<0.3 (<0.3)	2.0±0.02 (<0.3)	<3.0 (<3.0) 2.0±0.06[a]	67.8±0.4 (<3.0)

Results are presented as mean ±1 SD for each individual (triplicate analyses). Fasting values shown in parentheses are presented as mean ±1 SD for five separate experiments performed on each individual.

[a] The concentration of glucose in saliva 60 min after a rinse with a 10% (w/v) maltose solution.

subjects after 15 min and in all subjects in less than 1 h. Likely products of sucrose metabolism, i.e. glucose and fructose were not detected at any time interval. In the case of the maltose rinse concentrations had fallen to less than 3.0 µg/ml in 4 subjects after 30 min and in all 5 subjects by 60 min. In contrast to the sucrose rinse, however, substantial amounts of glucose were released, the subsequent loss of which followed the glucose rinse clearance curves. After 60 min, 3 individuals had glucose levels greater than the fasting levels but 2 had lower levels. Sorbitol clearance was the slowest and levels were up to 1,500 times higher than those for sucrose after 15 min with concentrations ranging from 6 to 930 µg/ml even after 1 h. Fasting levels were <3.0 µg/ml, the detection limit of the system.

Comparison of Foodstuffs

The clearance curves from saliva for glucose, sucrose, fructose and maltose for the chocolate bar, bread and banana in the 5 individuals are shown in figures 2–4, respectively. Bread consumption led to the appearance of maltose and glucose in saliva, bananas gave rise to sucrose, glucose and fructose and chocolate bars to sucrose, glucose, fructose and maltose. Salivary sugar concentrations 1 min after swallowing were highly variable with all three foods. With the chocolate bar, glucose concentrations ranged from 2.9 to 20.1 mg/ml, fructose from 0.9 to 9 mg/ml, sucrose from 19.6 to 99.3 mg/ml and maltose from 3 to 32.9 mg/ml; with bread, glucose was 0.2–0.6 mg/ml and maltose 5.8–10.9 mg/ml and with bananas, glucose was 1.2–5.5 mg/ml, fructose 1.2–4.8 mg/ml and sucrose 5.4–54.4 mg/ml. Levels at 60 min are shown in table 3. As with the mouth rinses, individual subjects showed the same predisposition to either rapid or slow clearance irrespective of the carbohydrate or the foodstuff. With the exception of glucose where the results were variable, in almost all cases, there were easily detectable amounts of sucrose, fructose and maltose residues from all three foodstuffs in the mouth even 60 min after ingestion. Chocolate bars and bananas produced high initial levels of glucose which were cleared rapidly at first and more slowly thereafter. In bread, however, where glucose is produced as a result of starch degradation by salivary amylase, low levels occurred and the clearance was slower. Despite the high glucose content of the chocolate bar, by 60 min the glucose levels are approximately the same for all three foods. The clearance of the monosaccharide fructose from saliva after ingestion of a chocolate bar and banana was similar. Generally, as was the case with the rinses, sucrose is removed the most rapidly. The disappearance of maltose varied considerably from person to person but in general the patterns of clearance were similar, initially rapid and slower thereafter.

Discussion

The time course of clearance of sugars from saliva can be represented in a number of ways: as a clearance rate, that is, the amount cleared per unit time and volume of saliva, as

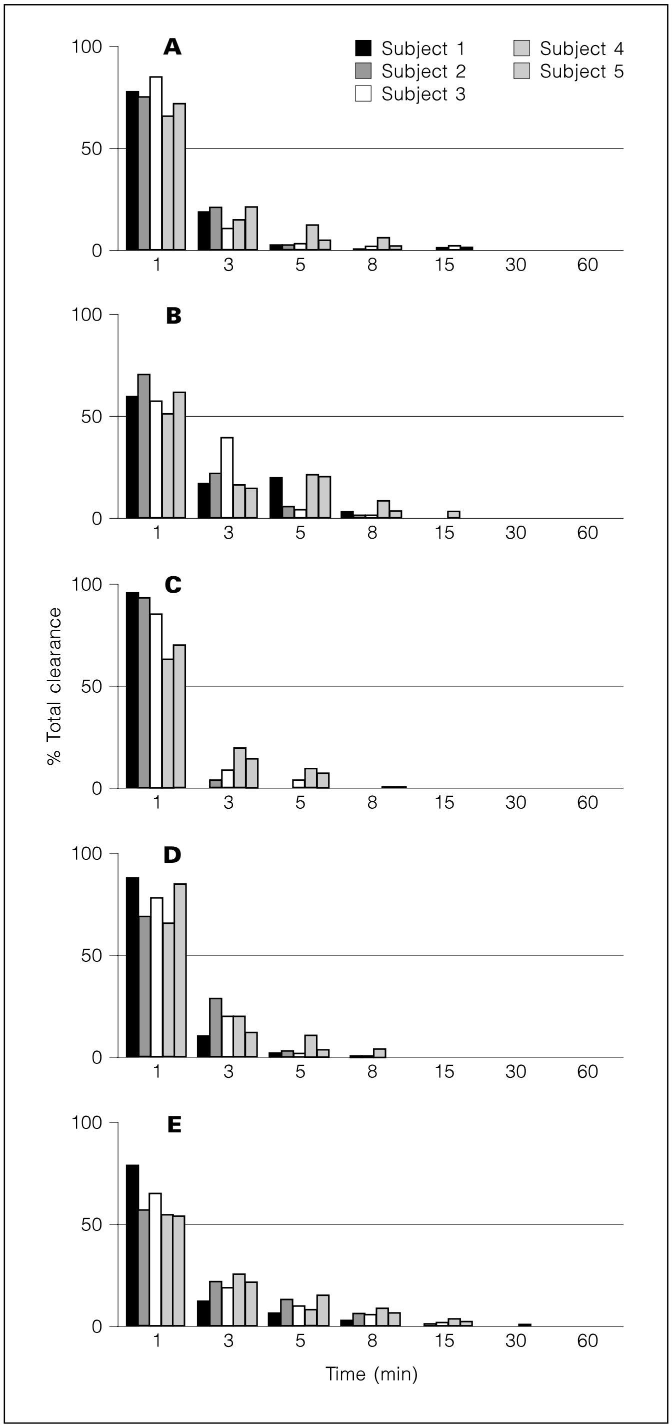

Fig. 1. Proportionate fall with time in the clearance of glucose (**A**), fructose (**B**), sucrose (**C**), maltose (**D**) and sorbitol (**E**) from whole saliva in subjects 1–5 following a 60-second rinse with 10 ml of 10% (w/v) solution. Results are expressed as a percentage of total clearance in 60 min. Bars represent the mean for each individual (quadruplicate analyses); SD <5%.

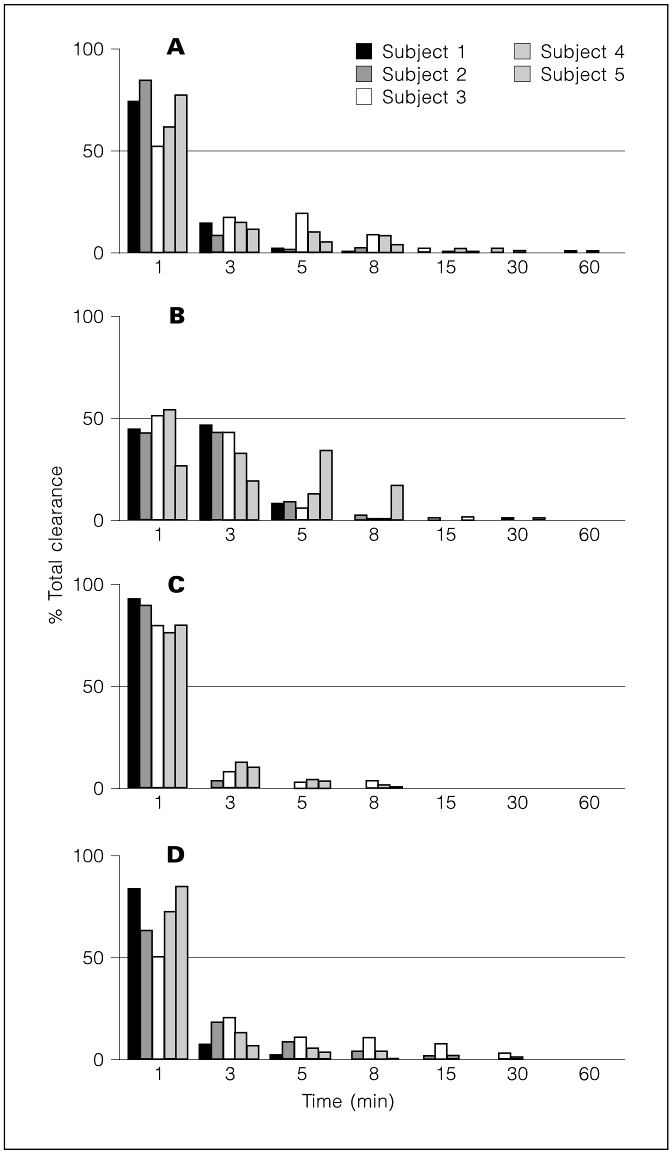

Fig. 2. Proportionate fall with time in the clearance of glucose (**A**), fructose (**B**), sucrose (**C**) and maltose (**D**) in a chocolate bar from whole saliva in 5 subjects expressed as a percentage of total clearance in 60 min. Results are presented as the mean for each individual (triplicate analyses); SD <5%.

the total sugar cleared in each time interval or, best of all, as a percentage of total clearance. To obtain a relation between the disparate units the sugar cleared in each time interval was therefore expressed as a percentage of the total cleared over 60 min. Whichever way it is expressed one phenomenon can always be discerned: with all sugars the clearance is high at first and falls rapidly with time, confirming the validity of Swenander Lanke's [1957] exponential equa-

Table 3. Sugar concentrations in saliva (μg/ml) from 5 individuals 60 min after food was administered

	Chocolate bar				Bread		Banana		
	G	F	S	M	G	M	G	F	S
1	126 (2.8)	1.3 (0.2)	1.4 (0.05)	3.4 (0.2)	144 (5.4)	31 (1.6)	52 (0.4)	16 (2.3)	10 (0.3)
2	9 (0.11)	39 (0.4)	21 (0.8)	10 (0.2)	11 (0.07)	8 (1.4)	16 (1.8)	1.8 (0.02)	1.0 (0.04)
3	11 (2.1)	7 (0.02)	31 (1.4)	10.7 (1.9)	14 (0.7)	10 (0.7)	9 (0.01)	1.3 (0.1)	1.4 (0.1)
4	29 (0.1)	1 (0.3)	14 (0.7)	8.2 (0.9)	41 (3.0)	26.5 (1.5)	18 (2.1)	5 (0.1)	1.8 (0.02)
5	1.3 (0.4)	1.3 (0.02)	1.2 (0.06)	8.1 (0.02)	72 (5.1)	1.4 (0.04)	31 (1.2)	2 (0.01)	1.3 (1.0)

Values are the mean of results of triplicate analyses from one experiment with SD in parentheses. Fasting values are those shown in table 2. G = glucose; F = fructose; S = sucrose; M = maltose.

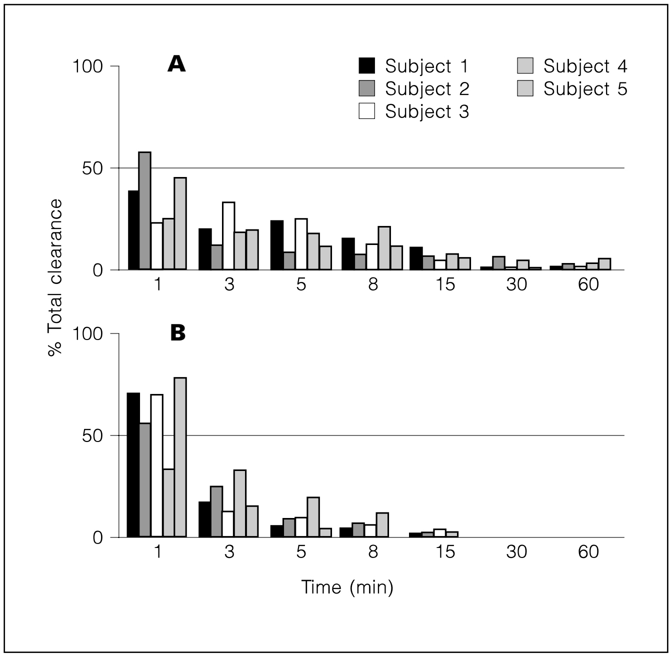

Fig. 3. Proportionate fall with time in the clearance of glucose (**A**) and maltose (**B**) in bread from whole saliva in 5 subjects expressed as a percentage of total clearance in 60 min. Results are presented as the mean for each individual (triplicate analyses). SD <5%.

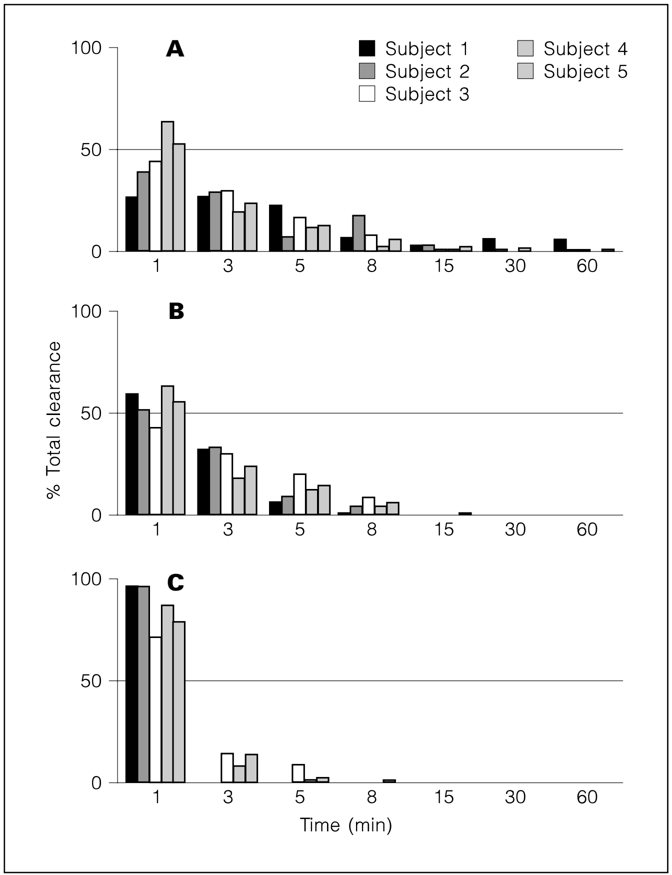

Fig. 4. Proportionate fall with time in the clearance of glucose (**A**), fructose (**B**) and sucrose (**C**) in bananas from whole saliva in 5 subjects expressed as a percentage of total clearance in 60 min. Results are presented as the mean for each individual (triplicate analyses). SD <5%.

tion for the rate of sugar clearance. The observation that salivary sugar clearance is characteristic of an individual is also in agreement with Swenander Lanke [1957] and is in accord with Dawes' [1983] concept that clearance is a function of parameters which vary within a narrow range for any given subject such as unstimulated salivary flow rate and the volume of saliva present in the mouth immediately before and after swallowing. Lagerlöf et al. [1994] reaffirmed that oral carbohydrate clearance is an individual property stable over an extended period of time, confirming the validity of the Swenander Lanke thesis.

The factors responsible for the loss of the five different carbohydrates used in the rinses will include physical removal by saliva flow and swallowing, metabolism by micro-organisms, adsorption onto oral structures and degradation by salivary enzymes. Sorbitol is poorly metabolised by oral bacteria and thus provides the clearest illustration of removal by physical factors alone. Sorbitol persisted at fairly high levels even after 1 h, a finding which supports the hypothesis that the oral mucosa and teeth are covered by an unstirred layer of saliva approximately 0.1 mm thick [Main et al., 1984; Dawes, 1989] and there is a slow loss of the sorbitol retained thereafter into freshly secreted bulk whole saliva. On the other hand glucose is metabolised rapidly by oral bacteria [Gough et al., 1996] and its removal will be caused mainly by a combination of both this and swallowing. The glucose levels detected therefore will be the net product of low levels of endogenously derived salivary glucose and residual exogenous glucose from the rinse. However, the fact that 3 of the 5 individuals still had glucose levels higher than the fasting values after 60 min and one was lower suggests that factors other than the rinse may be contributing to salivary glucose levels. Interestingly, in the same subject glucose levels after food intake fell below the fasting levels once again indicating that there may well be a bacterial source of salivary glucose such as degraded extracellular glucans in addition to dietary or secreted salivary sources. Retained food particles presumably would also serve as reservoirs of fermentable carbohydrates.

Food retention will depend on the nature of the food [Lundqvist, 1952] as well as the salivary flow rates and swallowing, chewing and tooth anatomy [Lagerlöf and Dawes, 1985; Speirs and Dean, 1989]. Chocolate bars and bananas produced high initial levels of glucose which were cleared rapidly at first and more slowly thereafter. In bread, however, the clearance is slower which may in part be due to its 'stickiness' and its retention in the mouth for a longer period, but may also be due to the slower breakdown of starch in saliva by salivary amylase. Results from the present study appear to be in agreement with those obtained by Edgar et al. [1975] and Bibby et al. [1986]. In studies of salivary carbohydrate they found that foods that were high in cooked starch content exhibited slow salivary clearance rates. Similarly, Kashket et al. [1991] showed that rates of clearance of carbohydrate from the saliva were slowest for the most retained foods; chocolate bars behaved as exceptions to this pattern showing high initial retention followed by rapid rates of clearance. In this study, however, carbohydrate residues from the three foods studied were still present in the mouth even 1 h after ingestion. This new finding may reflect the higher sensitivity of the carbohydrate assay system used and that fluoride was added to all samples immediately on collection as an inhibitor of glycolysis. The finding that glucose levels after 60 min were approximately the same despite the high glucose content of the chocolate bar is at variance with Goulet and Brudevold [1984]. They found the rate of glucose clearance decreased with increase in the level of glucose, albeit in rinse solutions. They suggest that with high concentrations, sugar diffuses into inaccessible locations and that clearance from these is slower, which in turn decreases the salivary clearance rate. The other monosaccharide, fructose, was not detectable in fasting saliva and clearance was incomplete after 1 h, probably because it normally undergoes slower bacterial degradation.

Comparison of the fate of the two disaccharides sucrose and maltose, however, gives further insight into the multifactorial nature of carbohydrate clearance from the oral cavity. Maltose, which is lost from saliva more slowly than sucrose, is generally also more slowly metabolised by the oral flora than is sucrose. Additionally, it is known that oral micro-organisms can utilise sucrose to form extracellular polysaccharides by extracellular enzymes (e.g. glucosyltransferases and fructosyltransferases). Accordingly, there is a metabolic fate for sucrose in addition to hydrolysis by invertase to glucose and fructose and utilisation of the products for glycolysis and intracellular polysaccharide production. The more rapid loss of sucrose seen by comparison of the curves for the sucrose and maltose rinses might be attributable to utilisation in glucan and fructan synthesis. However, if this were the case, the other products of the reactions, namely glucose and/or fructose, would be expected to be found in saliva, but this was not the case. Accordingly the mechanism must involve a process whereby either the glucose and fructose formed are immediately taken up by the cells which would necessitate vectorially oriented enzymes, or, sucrose transport across the cell membrane is substantially faster than glucose or fructose.

In contrast, in the case of maltose as early as the first post-rinse sample, i.e. 60 s after maltose had entered the mouth, exogenous glucose, presumably formed by maltose degradation, was detected in the saliva of all subjects. Within half an hour the maltose levels in saliva for all subjects were lower than the total glucose levels (i.e. exogenously derived from maltose plus that for endogenous sources).

The very rapid loss of sucrose is of the same order of magnitude as that reported by Oliveby et al. [1990] following a 30-second rinse with 20% (w/v) sucrose even though the sensitivity of their analytical procedure was substantially lower than in the present work. Sucrose is often regarded from animal and in vitro demineralisation/remineralisation studies, as well as from human experiments and epidemiological evidence, as being particularly cariogenic in the presence of a cariogenic microflora and susceptible tooth hard tissue. If its relatively rapid removal from saliva is, in part, a consequence of rapid utilisation or uptake by oral flora, studies on the mechanism involved are of especial relevance in furthering the understanding of its role in dental caries and elucidation of possible mechanisms for its inhibition.

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